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The Potential for Infectious Disease Contamination During the Artificial Insemination Procedure in Swine

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1. Introduction

The productivity of the swine confinement has been steadily increasing in the last years. According to data from [1], the largest producer of pork is China, which is responsible for producing 49.5 million tons and is followed by the European Union, United States and Brazil (22.5; 10.2 and 3.2 million tons, respectively). The increased productivity of the swine meat is mainly related to the use of technology in the genetics, nutrition, health and reproduction areas. The adoption of the artificial insemination (AI) in pigs from the 70-ies has been significantly contributing to the development of swine production [2].

AI has contributed to the increase of animal production, since it accelerates the dissemination of desirable characteristics from genetically superior animals. Worldwide, it is believed that 90% animals raised commercially are inseminated and this number is expected to further increase [3]. Thus, this biotechnology has been investigated in order to ensure best production indexes. These are supported by high rates of pregnancy and commercialization of the semen doses has been found, such as the creation of AI centrals in Denmark, Canada and Netherlands, which totalized exports to 35 countries in 2010.

The health issue and the difficulty of cryopreservation of the doses are considered as the main barriers to commercialization of doses from boar semen. Numerous studies have been carried out in order to develop efficient cryopreservation protocols, since this is the main method to ensure the maintenance of viable doses for a long period, as allowing for their transportation to long distances. Initially, the objective concerning AI was to obtain a better control over the sanitary conditions. However, it was noted that the considerable develop-

ment of this biotechnology was not accompanied by scientific knowledge related to the transmission of diseases. On one hand, the use of the artificial insemination has the great advantage in optimizing the use of the boar, whereas reducing the number of animals in the farm and consequently the costs of the management, medicines and animal acquisitions, the AI may function as a means diffusing pathogens, since there is no ideal sanitary control during the collection and manipulation of the semen. In this case, the AI using contaminated semen just maximizing spread of certain virus and bacteria since a single boar ejaculate can be used for insemination of various sows.

In this context, a considerable concern is assumed in relation to hygienic procedures in the semen manipulation process, especially in relation to semen destined for international market [3]. This fact is justified by the evidence of the possibility for transmission of some diseases via semen of swine. Among the possible agents that can transmit diseases are Aujeszky's disease virus, *classical swine fever virus* (CSFV), *african swine fever virus*, *porcine circovirus 2* (PCV2), *porcine reproductive and respiratory syndrome virus* (PRRSV), *porcine parvovirus* (PPV), *Chlamydia* sp., *Leptospira* sp, *Mycobacterium tuberculosis*, *Mycobacterium paratuberculosis* and *Brucella abortus* [4].

The effect from contamination of the boar's semen may represent a considerable economical loss to the producer. This occurs because the presence of the bacterial or viral agents in the semen leads to the loss of fertility and reduction of the semen quality in male, and embryonic/fetal death, endometritis and systemic infection in the inseminated females, thereby contributing to reduction in the size of the litters.

Although the routine addition of antibiotics (ATB) in the seminal diluents may even eliminate a high number of contaminant bacteria, most viral agents still remain alive. Therefore, a concern has been assumed in relation to those pathogenic agents. Despite the availability of studies concerning to antivirus, these ones are still not used commercially due to ineffectiveness of the action, especially related to high toxicity to sperm cells. Thus, the main control criteria AI are limited to veterinary communication, inspection by health agencies and control strategies such as vaccination, isolation and monitoring of animals [5]. Moreover, efficient routine tests for identification of contaminants in the semen samples still remain as a reality that is very far from the existent commercial farms.

Thus, this chapter aims to clarify some points referring to the potential for contamination by infectious agents during AI procedure in pigs, as well as to identify the main agents likely to be transmitted by this biotech, which can contribute to reduced fertility of the animals, besides the possible control measures that should be performed in order to reduce the dissemination and effect of those pathogens on animals.

2. Main risky points during Artificial Insemination (AI)

IA is a commercially widespread technique worldwide. Therefore, the procedure for collection and manipulation of the semen besides the AI itself must be carefully accomplished in

order to ensure that AI will not represent a risky factor for transmission of infectious diseases. Although there are wide variety of diseases that could contaminate the semen and consequently the inseminated female, the significance of a particular disease will vary according to epidemiological parameters and geographical localization of the farm. Even the risk for disease transmission is not the same in different countries of the world. Hence, the concept of pathogen-free centrals has become a common cause, since it is possible to obtain the pathogen-free semen either in countries that are free and in those that are not free from certain disease, according to definition by World Organization for Animal Health (OIE).

Guérin and Pozzi, (2005) [6] suggested that diseases able to cause negative impact on AI can be evaluated according to health risk as follows: a) Diseases that were eradicated within a country or continent, such as the Classical Swine Fever in Brazil; b) diseases to which there is already an integrated program for control in AI, such as the CSFV or Aujeszky's disease, which implies a negative state of the donor boars; c) diseases that are considered as likely to be transmitted by AI, such as diseases associated with PCV2, PPV and transmissible gastroenteritis, which are neither controlled nor associated with prophylactic measures routinely adopted.

The seminal contamination may be classified as extrinsic or intrinsic. The first case occurs when contamination occurs through an external source, such as feces or contaminated materials used during semen collection or processing. The intrinsic contamination occurs due to viral infection that can be systemic or local, as occasioning viral elimination through testicles, accessory or preputial glands [6]. Thus, it can be indicated that the main risk points for contamination of the semen can occur at the semen collection stages, in semen manipulation, or in artificial insemination procedure according to sanitary conditions of the farm.

Before the semen collection procedure, all utensils to be used and specially the material in contact with the semen must be sterilized according to routine hygienic procedures and equipments available at each AI station. The use of dry heat (ovens), moist heat (autoclave) and radiation (ultraviolet) are most suitable for sterilization. These materials include the collecting funnel and the collecting glass where the semen will be stored until the moment of dilution. Because accidents may occur during the collection procedure, it is advisable to build up a stock of sterile materials ready to be used in the case of contamination during the procedure.

The animal' prepuce is usually contaminated by a wide variety of infectious agents, as reported by some authors [7,8,11]. Thus, the occurrence of agents such as *Corynebacterium suis*, *Arcobacter spp.* and the Aujeszky's disease virus (ADV) in the ejaculate of the infected animals becomes a real possibility to be considered. Therefore, the examination of the semen-donor animals prior to collection of the semen is essential in order to ensure the sanitary quality of the semen. However, this previous evaluation may be not completely effective. This is due to the fact that certain viral agents, such as ADV which causes the Aujeszky's disease, have the peculiar ability to establish latency in the reproductive organs, therefore causing contamination of the semen although the animals remain serologically negative for those diseases [9]. Other viral agents such as PCV2 and PRRSV have a determinated sero-conversion period over which the agent will be eliminated through semen. Even when the

animal is serum-negative for the infection [10], this elimination may still occur under continuous or intermittent way during the months after infection [12]. Another measure that can be taken in order to reduce contamination is the elimination of the first ejaculatory jets, that are characterized by high microbial contamination, therefore obtaining a better quality of the semen. The environmental contamination is also a factor to be considered during the collection procedure. Thus, the sanitary procedure of either male' preputial region and the dummy sow used for collection are routine procedures to avoid an eventual contamination of the semen outside the animal' body.

When processing the semen, certain hygienic precautions should be taken during its dilution and straw filling in order to prevent contamination. Regardless of the viral source, the storage and manipulation conditions are fundamental to predict the potential risk for contamination of the semen. It is known that the fresh semen is favorable to preservation and dissemination of the virus between species [6]. Therefore, the places where they will be in contact with the semen should be thoroughly sterilized to prevent contamination. There is a false impression that the antibiotics present in diluents can prevent bacterial contamination, but this detail should be cautiously considered, since the antibiotic doses contained in the seminal diluents might contain only the bacterial proliferation and are ineffective against some specific strains and virus [13].

Another factor to be considered is the insemination procedure itself. During AI, the main contaminative source is the feces, which is contaminated mainly by *Porcine sapelovirus*. Therefore, the care related to washing of the perianal region should be reinforced in order to avoid contamination. Other agents which may be found in the feces such as *Escherichia coli*, *Salmonella sp.*, *Rotavirus A*, Porcine Adenovirus, when introduced into uterus through insemination pipette, may cause the infection of the uterus and consequently leads to reduction of fertility and litter size.

Concerning to the intrinsic contamination forms, many local and systemic diseases may move towards the reproductive tract and are transmitted via semen. Those diseases can be divided into viral and bacterial diseases. In relation to viral diseases, some have more potential risk for transmission, as presented in Table 1.

Most diseases that affect the reproductive tract and are caused by viral agents rather provoke classical clinical signs that serve as parameters for isolation of the animals besides avoiding the animal reproduction. However, some diseases may be transmitted via breeding, even when the animal shows no clinical signs becoming an even greater problem, since it is not possible to identify the infected animal [14]. It is believed that the period over which there is greater release of viral load is when the animal shows the clinical stage of the disease [15].

In cases of the appearance of clinical signs, generally the males are not used for reproduction. Furthermore, the males usually refuse to make the natural mating during those situations. In addition, there is the guarantee of the reduced risk for transmission the disease, unlike when there is no apparent infection. Finally, the insemination of the sows infected with a semen does not necessarily result into contamination of the female and the onset of the clinical disease in

the same one. Thus, the complexity of the conditions required for establishment of this process were experimentally proved.

Agent	Virus Isolation in Semen	Potential risk for contamination
Porcine adenovirus	+	Low
Aujeszky diseasevirus	+	+
African swine fever virus	+	+
Blue eye disease virus	+	+
CSFV	+	+
<i>Porcine sapelovirus</i>	+	+
FMDV	+	Low
Influenza virus	+	Low
PCV2	+	+
PPV	+	+
PRRSV	+	+
Reovirus	+	Low
SPV	ND	+
SVD	+	+
TGEV	+	Very Low

Table 1. Presence of the viral agents in semen of pigs and the risk for their transmission through AI (Adapted from [14] and [6]. ND: No data; CSFV: *classical swine fever virus*; FMD: *foot and mouth disease virus*; PCV2: *porcine circovirus 2*; PPV: *porcine parvovirus*; PRRSV: *porcine reproductive and respiratory syndrome virus*; SPV: *swine papilloma virus*; SVD: *swine vesicular disease virus*; TGEV: *transmissible gastroenteritis virus*.

Depending on pathogen, other less important forms of the seminal contamination should be considered. Among them, it can mentioned the transmission through aerosols, urine, fomites, people, vectorial insects, birds and wild mammals [5].

Finally, for complete determination of the level for the disease transmission risk in a farm, the hygienic and sanitary standards adopted in this farm should not be disregarded. In practice, it is observed that farms which do not provide effective vaccination programs and present failures in sanitary practices are more subject to contamination of the animals. The animals under these environmental conditions face a constant challenge.

3. Major contaminants of semen during AI procedure

With the high spread of the AI technique, semen has become an important vehicle for dissemination of pathogenic agents, either by previous infection of the male' reproductive tract

or by contamination of the ejaculate through inadequate hygiene of the person collecting, direct contact with animal's feces or even the use of contaminated diluents. Several agents such as viral or bacterial, may be present in semen or may contaminate it after ejaculation.

3.1. Bacterial contaminants

Naturally, the pig's fresh semen contains approximately 10^4 to 10^5 bacteria/ml [16]. Although those bacteria are not pathogenic, they present spermicidal effect, especially when they are present at high concentrations [17]. To aggravate the situation, the majority of the bacteria which may be present in semen have innate or acquired resistance to antimicrobial agents added to diluters of the semen [18]. Many antimicrobial agents may still have their optimum action impaired by environmental conditions, such as the temperature [19]. Therefore, even with addition of antibiotics, the bacterial transmission through AI is a situation that may occur.

Common bacteria which are associated with infections of the sows' genital tract and are possibly transmitted via semen are presented below.

3.1.1. *Leptospira* spp.

Spirochetes of the *Leptospira* genus are the agents causing leptospirosis, a disease mainly characterized by reproductive disorders. In serological study conducted in Brazil, Favero et al. [20] (2002) observed that the most prevalent serovars associated with Leptospirosis are: pomona, icterohaemorrhagiae, copenhageni and tarassovi. The disease has worldwide distribution and leads to infertility of the animals [21].

The main route for elimination of the Leptospire is through urinary system [22]. However, they may be present in the infected animals' semen, as causing the infection of the female and can lead to reproductive complications during the bacteremia phase, inclusive abortion [23]. The bacteria can persist in the kidneys and reproductive organs of both males and females, therefore facilitating the dissemination of the disease in herd, requiring an early diagnosis of the disease.

To fill out this need, the tests for antibody detection by serology are effective. However, for serotyping the Leptospire is necessary to consider other diagnostic techniques. Attention should be given to the vaccinated seropositivos animals, since the antibodies are likely due to vaccinal reaction. For detection of the agent, molecular tests can be performed by PCR and immunoassay such as the direct immunofluorescence [24, 25].

In the case of a positive diagnosis, even with few affected animals, the control must be taken by adopting the following criteria: management, fight against rodents, vaccination and drug treatment in order to prevent the dissemination of the disease in the herd. Considering that *Leptospira* are sensitive to a wide variety of antimicrobial agents, the treatment associated with vaccination and sanitary measures provides an effective control of the disease [26].

3.1.2. *Mycobacterium* sp.

Bacteria of the *Mycobacterium* genus are agents causing tuberculosis, a disease characterized by provoking granulomatous lesions in various organs. In pig herds, *Mycobacterium avium* is the most prevalent species, but infections caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis* can also occur [27]. Although pulmonary tuberculosis is the commonest form, the dissemination of the infection by several other organs can occur in a form so-called milliary tuberculosis [28].

When the disease appears under milliary form, the granulomatous lesions may be present in the reproductive organs with caseous necrosis with areas of calcification in the testis and epididymis, therefore the elimination of the microorganisms by semen will occur [29, 30].

Those lesions associated with confirmatory tests, by using special colorations to identify the alcohol-acid resistant bacilli are sufficient for definitive diagnosis of the disease. For characterization of the species, the PCR technique has been used since the isolation of the mycobacterium strains is considered as laborious procedure [31, 32].

The possible sources of infection can be determined by characterization of the agent. Thus, the complete and definitive diagnosis is very important to the control. Moreover, the issues concerning the farm hygiene are factors to be considered because the exposure to feces are the main factor for infection and dissemination of the disease [33].

3.1.3. *Brucella suis*

The etiologic agent of brucellosis in pigs is the *Brucella suis*. The disease is characterized by high morbidity and reproductive disorders such as abortion, endometritis and placentitis in females and orchitis, changes of accessory glands, libido loss and infertility in male [34]. Abortion has been observed at 17 days after female cover with males which are positive for *B. suis* in semen. Infertility in animals is mainly due to the involvement of testicular structures and lack of libido in the infected animals. The cases of contamination of the accessory glands are even more critical, since the animals remain fertile and can disseminate high loads of *B. suis* in semen during prolonged periods.

It is an extremely important disease for countries of the South America, Asia and Africa, where it is totally widespread. In the countries of North America and European Union, the prevalence is low or the disease has been eradicated [19]. The main route for elimination of brucellosis in farms is the genital arising from a positive male which eliminates the microorganism in the semen. Bacteria reach the reproductive organs after invasion of the lymph nodes followed by bacteremia [35]. In male, the infection may persist throughout life. Thus, it is necessary to eliminate the positive animals to prevent the dissemination of the disease.

In the case of positive farms, the control procedures should be performed. Among them, the sanitary break after the elimination of the positive animals and the monitoring of the reproducers' serological profile has proved to be effective for elimination of the agent of the herd. Although, nowadays, the sanitary conditions in commercial farms and the agility of the de-

definitive diagnosis have evolved considerably, some pathogens have generated insignificant infection levels, such as the case of *B. suis*.

The definitive diagnosis is accomplished through isolation of the agent. Although very specific, it is complex and expensive, as requiring efficient and alternative methods. Serology can be used but must be associated with confirmatory tests such as rivanol, 2-mercaptoethanol and complement fixation. Another possibility is the molecular diagnosis by PCR [36].

3.1.4. *Chlamydia* sp.

Chlamydiosis is a disease with worldwide distribution that affects several species of mammals and especially birds. The main species causing disease is *Chlamydia psittaci* [37] and it is associated with pneumonia, conjunctivitis, enteritis, arthritis, pericarditis, orchitis and uterine infections, as being the last two related to cases of perinatal abortion and stillbirths [38].

The microorganism can enter through digestive, respiratory or venereal via and multiply in the epithelial cells that are carried by macrophages and disseminate by the chain of regional lymph nodes and remaining unapparent, but sometimes causes diseases in organs nearby routes for entrance of the agent. In genital infections, the semen may be contaminated and it is responsible for birth of the weak (which eliminate the bacteria during long period, therefore they are an important vehicle for horizontal transmission of infection [39].

For diagnostic purposes, several serological tests can be performed and the agent detection can be performed through the isolation and PCR. However, fecal sample has little diagnostic value because studies have demonstrated the presence of *Chlamydia* in healthy pigs [40].

Although the main dissemination sources to be the asymptomatic animals, the infected animals showing clinical signs of disease should be isolated and treated [41]. It is important to avoid the contact of pigs with birds and other species that are susceptible to Chlamydiosis.

3.2. Viral contaminants of semen

Recently, a high number of viral agents have been detected in the semen of pigs. Those agents are mainly associated with reduction of the animal's reproductive performance and fertility problems [6]. Usually, the infections by virus is source of major concern to the swine producer than bacterial infections. This fact is due to characteristics of the viral agents, which can be eliminated at high loads before the first signs of the illness or when the signs are mild or unapparent, as causing a significant epidemiological problem. However, it is believed the high virus load to be only removed via semen during viremia. During this period, the breeding male presents clinical signs, therefore it is generally removed. In any way, the control procedures are hindered because the animals can continue to eliminate the virus after disappearance of the signs. In addition, the efficacy of the available commercial antiviral products are not commercially proved and may exhibit a high toxicity level to the semen.

Some major viral agents, which may be present in the genital tract and eliminated through semen, are summarized in the sequence.

3.2.1. *Porcine reproductive and respiratory syndrome virus (PRRSV)*

The PRRS is a disease characterized by reproductive failures and respiratory diseases caused by PRRS virus. After infection, the virus elimination period can last up to three months [12] which enables the virus to disseminate regionally, nationally and internationally through transit of the infected animals.

During this period, the discharge can occur by several routes, as the semen being among the principal ones, what results into infection of the female and reproduction failures. In the body, the virus multiplies in macrophages and establishes the first viremia and can reach various organs and systems, as including the reproductive tract. In female, it crosses the placental barrier and results into miscarriages and birth of weak piglets, which will be disseminators of the virus in herd [42]. The virus can be eliminated in the semen even in absence of the viremia and in presence of the neutralizing antibodies [43].

The changes in the semen contaminated with PRRSV present individual characteristics, with substantial quality loss through reduced motility, increased percentage of abnormal acrosomes and increase of the spermatozooids with altered morphology [44] as those spermatic pathologies being an indication for infection with PRRSV.

Complementing the clinical signs and spermatic changes, the serological techniques are effective for definitive diagnosis. However, those techniques indicate exposure to the agent without the guarantee of the presence of infection and the vaccinated animals have higher levels of antibodies, what may lead to false-positive results [45]. The viral isolation, RT-PCR and immunohistochemistry techniques are employed for the diagnosis of PRRS in which the virus is detected [46-48].

To control the disease, the commercial vaccines are effective in reducing the viral load from the infected animals [49]. In countries where there are no reports of the disease, the monitoring programs of the entry of animals and semen should be well established and rigid.

3.2.2. *Aujeszky diseases virus (ADV)*

The ADV is the target of numerous control and eradication programs, and many of those programs have already achieved success and the aujeszky-free status. The ADV is the causative agent of the Aujeszky disease (AD), that is characterized by clinical respiratory signs and nervous and serious reproductive disorders [50].

The ADV had been isolated from prepuce and detected in the semen of the reproducers [51]. In 1984, [52] carried out a study with experimental infections. They observed that testicular degeneration and decreased semen quality due to fever of the infected animals are frequent in ADV-positive animals.

The DA suspicion is raised by symptoms, but laboratory tests are necessary for the definitive diagnosis, since the virus can be detected in tissues or secretions of the animals through virological diagnosis. Serologic tests can be used, and ELISA is the most indicated because it can differentiate the antibodies proceeding from the immune response of the vaccines with antigenic markers from those ones infected with the field virus [53].

Eradication through vaccination, removal of the infected animals and depopulation of the positive farms have achieved success in several countries [54]. However, care must be taken with the wild pigs which are PRV reservoirs [55].

3.2.3. *Classical swine fever virus (CSFV)*

The virus of the classical swine fever belongs to Pestivirus genus. It is highly contagious and causes the classical swine fever (CSF), with mortality rates ranging from 80 to 90% and leads to a framework of generalized bleedings. The contact with wild animals and infected food and the transit of animals are the main forms for CSFV dissemination. Therefore, the marketing of semen for AI is considered an additional hazard [56].

In an experimental study, van Rijn et al [57] (2004) observed the presence of CSFV in pigs' semen at 3 days after infection. The elimination continued intermittently until the end of the experiment (18 days), as proving that artificial insemination can be a risk factor for transmission of the disease.

Due to importance of the disease, the clinical suspicion should be investigated by laboratory techniques. While virus isolation is the gold standard, other tests such as ELISA and RT-PCR can be used for definitive diagnosis [58, 59]. The tonsils, spleen, pharyngeal and mesenteric ganglions are the favorite organs for sending to laboratories.

In the case of diagnostic confirmation, several procedures should be taken in order to prevent the virus from spreading through the region. The sacrifice of the positive animals, the prohibition that animals and semen to transit in the region as well as the installation of sanitary barriers are actions for controlling the outbreak. Another control procedure is vaccination, however only attenuated alive vaccines are available, as hampering the differentiation between vaccinated and infected animals [60].

3.2.4. *African Swine Fever Virus (ASFV)*

The African swine fever virus is the causative agent of the african swine fever (ASF), that is a highly contagious and lethal disease characterized by a clinical picture similar to that of the classical swine fever [61, 62]. The epidemiological characteristics of the disease include the potential for rapid dissemination through direct and indirect contact as well as a natural transmission via arthropods and wild Suidae.

The virus of the African swine fever was isolated from the semen of infected pigs [6, 57]. The virus elimination through bodily secretions can last up to 70 days in persistently infected animals [63], which are the main villain in dissemination of the virus in herd.

Besides the epidemiological importance, the persistently infected animals are the major obstacle to diagnosis because they present less severe clinical signs, as requiring confidential laboratory tests in order to establish a reliable definitive diagnosis of ASF as well as to provide relevant information about the time of infection in order to successfully support the control and eradication programs [64]. The viral isolation is an important tool for diagnosis,

however it is a laborious and very slow procedure. The PCR technique has good sensitivity and specificity and is a faster alternative for detection of the virus [61].

Because of the unavailability of the vaccine against ASFV, the control strategies involve circulation restrictions, biosecurity and stamping out [65]. In Spain, the successful ASF eradication has been associated with the screening and removal of the persistently infected pigs [66].

3.2.5. *Porcine circovirus 2*

The *porcine circovirus 2* (PCV2) is the causative agent of the porcine circovirus and may present six different clinical syndromes, that are the multisystemic weakening syndrome (PMWS), dermatitis and nephropathy syndrome, the reproductive, respiratory, digestive and nervous failures [67, 68]. However, PMWS and the reproductive failures are only ones caused by PCV2 without the presence of cofactors [69].

In the aborted, stillbirths and/or mummified fetuses, the inflammatory changes can be observed in the myocardium associated with depletion of lymphoid tissues [69]. In those situations, the probable infection source of the females is the contamination of the positive male's semen. Opriessnig et al. [70] (2006) demonstrated through IHC the presence of the virus in cells of the testis, epididymis and accessory glands.

Besides IHC, the PCV2 can be detected by hybridization in situ (HIS) and PCR [71]. The virus isolation can also be used. However, the virus produces no cytopathic effect in the cells, therefore it is necessary to detect the viral antigen by immunofluorescence or immunochemistry.

Recently, Blomqvist et al. [72] evaluated the reduction of the viral load in semen after single layer centrifugation followed by a swim-up. They observed a reduction higher than 99% in the semen samples. Furthermore, the commercial vaccines have been very effective for controlling the disease in infected herd.

3.2.6. *Porcine parvovirus*

The porcine parvovirus (PPV) has worldwide distribution and is responsible for reproductive failures that are characterized by embryonic death, fetal mummification and stillbirth [73]. PPV can be a non encapsulated virus. It is resistant to adverse environmental conditions, which facilitates its dissemination. In addition, there may be venereal transmission of the virus from the infected semen. Besides the semen, the virus can be detected in testis, in the scrotal lymph nodes and in epididymis [6].

The techniques for virus detection are diverse and the direct immunofluorescence and PCR are the most commonly used methods. Serology can also confirm the presence of the anti-PPV antibodies. Although the virus isolation may be necessary to detect the viral sample, the fetal tissues are toxic to cellular cultures, therefore limiting the use of this technique in some situations [74].

The PPV-induced reproductive failures can be prevented by making sure that the development of the females' immune response occurred before conception. The immune response can result from natural exposure or from vaccination which is a common practice and performed at least annually [74].

4. The interference of diseases in AI efficiency

In swine, the efficiency of the AI programs is related to higher pregnancy rates, reduced estrus repetition rates and high number of the piglets born per litter. However, to obtain reproductive efficiency, several parameters must be optimized such as the animal nutrition, thermal comfort, skilled labor, genetics and mainly the sanitary aspect. This last factor is fundamental for the herd of the animals involved in reproduction to be totally free from disease and properly immunized against the most common diseases that can lead to reproductive disorders.

Therefore, the assurance of the animals' health is extremely important to ensure the absence of contamination of the animals' semen. From the scientific evidence that the presence of a virus or bacteria in the male' semen may reduce the fertility rates in the male and the female to be inseminated, the animal contamination by infectious diseases should be avoided.

The direct impact that occurs in males is mainly related to reduction in the sperm quality and numbers of doses produced. The reason for the impairment of the semen quality is not totally elucidated. Therefore, the losses to the farmer is considerable because it is often necessary to discard the boar because irreversible degenerative changes at testicular and epididymal levels by diseases that lead to fever for prolonged periods.

Solis et al. [75] reported that the experimental infection of the animals with porcine rubulavirus (PoRV), which causes the blue eye disease (BED) was able to cause orchitis in animals, as also affecting the portion of the epididymis. The virus was detected in the semen, either in the sperm and jell fraction. Those researchers observed the ability of this virus to cause severe alterations in sperm concentration, motility and morphology of the infected animals'. Those changes were aggravated according to the time of the sperm storage. Taking into account that the virus does not affect the adjacent glands, the seminal volume remained unchanged. The changes in other parameters occurred due to inflammatory event of the virus on the spermatid ducts, as leading to loss of the spermatid cells. Most viruses behave like aforementioned, however there are still many doubts about the extent of the virus interaction with the spermatozooids. Thus, future molecular studies are needed to elucidate the mechanism of those diseases.

In females, reports suggest that PRRSV was previously isolated from ovaries of infected animals, particularly locating in either granulosa cells layer and theca cells layer in atretic follicles of those animals. However, there are no reports of this virus in sows' oocytes [76, 77] neither the viral effect on their development ability. In infections associated with PCV 2, the oocytes collected from serum-positive animals for infection did not show to be positive for

the presence of the virus. Thus, the contamination via oocytes in naturally infected animals is not a natural route [78]. Yet this author and collaborators found that the virus can adhere firmly to either oocyte-cumulus complex and pellucid zone of embryos at the initial development stage despite not affecting the embryonic development.

At embryonic level, it has been demonstrated that the replication of some viruses can occur in the embryonic cells. In this context, the Pellucid Zone (ZP) of the embryos acts as a barrier protecting the embryo against viral agents. Therefore, after disruption of the pellucid zone at stage of the hatched blastocysts, some viruses such as the classical swine fever virus and PCV-2 can replicate in embryonic cells as carrying a deleterious effect, especially in embryos produced in vitro [79, 80]. The greatest weakness of the embryos produced in vitro may be due either to a thinner pellucid zone of those embryos [81] and greater exposure to laboratory conditions and culture media that can act as contamination sources. It is known that ZP of pig embryos is much stickier than that of cattle, although the reason for this fact to be not known [82]. It is believed that lower-sized virus (20 -26 nm), such as *porcine circovirus 2* and porcine parvovirus could even surpass the ZP of embryos produced in vivo by promoting contamination of the embryonic cells [13, 80]. However, this issue is still controversial and further studies are still needed.

Thus, it is expected the contamination of the embryonic cells of the ZP-unprovided embryos will depend mainly on nature of the virus, of the embryonic development stage and the presence of viral receptors expressed in target cells [82]. Furthermore, the ZP- unprovided embryos that are produced in vitro are much more sensitive to viral contamination and, independent of the nature, they represent a real source for contamination of the animals mainly by diseases caused by virus. Finally, the disinfection of the swine embryos by using washing and treatment with enzymatic combination rather represents a reasonable alternative for programs of the in vitro embryonic production [78].

5. Possible control procedure to be performed

Because the differences in the prevalence rates of the diseases among countries and even regions, the control strategies will differ according to incidence of each disease. Therefore, the policies for eradication, vaccination and isolation of the animals in farms are very dependant on the types of disease the animals would be more exposed.

The preventive procedures against transmission of infectious diseases via semen depend on the control routine. The AI must be understood as a contaminative potential for swine females, since it is a vehicle for disease transmission. Thus, the insemination centers should be regularly controlled and monitored according to specific criteria. However, even before considering the potential for contamination through semen, it is necessary to pay attention to the possibility for disease introduction through acquisition of a living animal. Thus, some practices such as the introduction of animals which are serologically negative or animals proceeding from seronegative herds and to avoid the contact of the animals pertaining to insemination center with external people are essen-

tial to prevent the introduction of diseases. After acquisition and routinely on farms, the male considered as potential disease disseminator only will be introduced in the semen collect program after a quarantine period, during which he remains isolated and under observation in order to verify if there is any abnormality sign. After introduction of the male in the breeding herds, it should be daily observed for signs indicating clinical disease. In the case of any abnormality in those animals, semen collections should be immediately interrupted. This method is highly effective for controlling the diseases that present evident clinical signs.

Diseases that have high dissemination potential and can be transmitted via aerosols, such as PRRS and AD, as might cause high losses in the farms should be monitored through periodic serological tests. Another important factor to be considered is the hygiene in the farm. The cleaning and disinfection of the installations before the entry of the animals, besides respecting the sanitary break period, are essential to prevent the dissemination of the pathogens.

The effective use of the antimicrobials to control contamination in diluents can act effectively, as minimizing the action of the bacterial and fungal agents [82]. Currently, there are many antimicrobial agents commonly used in seminal diluents such as aminocyclitols, aminoglycosides, beta-lactams, lincosamides and macrolides. However, these agents do not prove to be totally effective against some of the disease causing agents [18]. In routine of the farm, the antibiotics are added to seminal portions, as expecting high level of accidental contamination in the attempt to reduce the proliferation of bacteria.

Although the availability of the studies including the use of the antiviral drugs to inhibit the replication of the virus in the male's reproductive tract [83], the control of viral pathogens still needs to be better understood and will follow the pathway similarly to the one accomplished for bacteria [5]. Unlike the semen treatment with antibiotics, which can reduce or prevent the dissemination of venereal diseases caused by bacteria, the antiviral agents used to prevent contamination of the semen are not adopted in the swine IA industry. Therefore, many countries have adopted other successful strategies in maintenance of the specific viral pathogen-free centers. In those centers, the main control strategies are based on animal monitoring program for specific viruses. The animals are serologically evaluated and the serologically positive animals are readily eliminated from breeding herds [6].

As previously mentioned, recently Blomqvist et al. [72] observed a reduction higher than 99% at PCV2 concentration in semen samples. This new technique has shown to be effective against several other viral agents, which are present in samples of the human semen and other domestic species' [84-87]. Thus, this method represents a promising alternative for the control of viral contamination in the pigs' semen.

Another possibility for controlling the dissemination of diseases would be the programs for vaccination against the main agents that can be carried by semen and lead to diseases in

sow. However, there is no vaccine against some of those agents such as the ASF case, therefore making necessary the individual control methods as previously detailed.

6. Conclusion

The increasing tendency of the international trade in pigs' embryos and gametes has been stimulating an intensive investigation of the disease transmission via semen and porcine embryos. There are numerous diseases, both bacterial and viral causes, which are linked to transmission via boars' semen. In particular, each agent provides a type of interaction with gametes and has a specific site of action, which hinders the establishment of specific control procedures. Thus, despite the promising researches, many conclusive studies are required to ensure the innocuousness of the gametes from the infected animals. In addition, the effective and rapid diagnostic methods and effective control procedures should be developed and optimized in order to allow the access to swine farmers.

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